Stimulation, by vasopressin and other agonists, of inositol-lipid breakdown and inositol phosphate accumulation in WRK 1 cells

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WRK 1 cells were labelled to equilibrium with 2-myo-[^{3}H]inositol and stimulated with vasopressin. Within 3 s of hormone stimulation there was a marked accumulation of ^{3}H-labelled InsP_2 and InsP_3 (inositol bis- and tris-phosphate), but not of Ins$P$ (inositol monophosphate). There was an associated, and rapid, depletion of ^{3}H-labelled PtdIns$P$ and PtdInsP_2 (phosphatidylinositol mono- and bis-phosphates), but not of PtdIns (phosphatidylinositol), in these cells. Some 4% of the radioactivity in the total inositol lipid pool of WRK 1 cells was recovered in InsP_2 and InsP_3 after 10 s stimulation with the hormone. The selectivity of the vasopressin receptors of WRK 1 cells for a variety of vasopressin agonists and antagonists revealed these to be of the V_{1a} subtype. There was no receptor reserve for vasopressin-stimulated inositol phosphate accumulation in WRK 1 cells. The accumulation of inositol phosphates was enhanced in the presence of Li$^+$ ions. Half-maximal accumulation of Ins$P$, InsP_2 and InsP_3 in vasopressin-stimulated cells was observed with 0.9, 3.0 and 3.6 mM-Li$^+$ respectively. Bradykinin and 5-hydroxytryptamine also provoked inositol phosphate accumulation in WRK 1 cells. The effects of sub-optimal concentrations of bradykinin and vasopressin upon inositol phosphate accumulation were additive, but those of optimal concentrations of the hormones were not.

INTRODUCTION

Many hormones and neurotransmitters that influence intracellular events via Ca^{2+}-mediated protein phosphorylation share a common mechanism of signal transduction which involves receptor-mediated PtdIns(4,5)P_2 degradation and the mobilization of InsP_3 and diacylglycerol as second messengers (see refs. [1,2] for reviews). Such ligands include vasopressin, which interacts with specific V_1-receptors in liver [3–5], vascular smooth msucle [6] and the sympathetic nervous system [7] to provoke inositol-lipid breakdown and intracellular Ca^{2+} mobilization.

Monaco and her co-workers [8,9] have previously shown that vasopressin enhances protein accumulation and the incorporation of [^{14}C]acetate into lipids in WRK 1 cells, a cloned cell line derived from a chemically induced mammary tumour. These effects were associated with a marked stimulation of [^{32}P]P$_i$ incorporation into PtdIns [10–12]. In the preceding paper [13] we demonstrated that vasopressin binds to a single class of V_1-receptors on WRK 1 cells. It is now clear that V_1-receptor-mediated PtdIns labelling in liver [4,5,14–20] and other tissues (see refs. [1,2] for reviews) is associated with the prior degradation of PtdIns(4,5)P_2 and the associated accumulation of the Ca^{2+}-mobilizing second messenger InsP_3.

In the present study we have characterized the accumulation of inositol phosphates provoked by vasopressin and other ligands in WRK 1 cells. We provide evidence that several hormones share a common mechanism of signal transduction, involving PtdIns(4,5)P_2 degradation, in these cells.

MATERIALS AND METHODS

Cell culture and incubation

WRK 1 cells were established and grown as described in the previous paper [13], except that myo-[2-^{3}H]inositol (2 μCi/ml) was included in the culture medium for the entire period during which the cells were grown on 35 mm-diameter plastic Petri dishes before experimentation. After 4 days in the presence of myo-[2-^{3}H]inositol, when the cell density was about (5–7) × 10^5 cells/dish, the culture medium was aspirated and replaced with 1.5 ml of non-radioactive and serum-free medium. The cells were incubated for a further 1 h at 37 °C before the medium was aspirated again and the cells washed with 2 ml of PBS. The cells were then bathed in 2 ml of fresh PBS and maintained at 37 °C for the rest of the experiment. Where appropriate, LiCl was added to incubations 10 min before the addition of hormones or other ligands. When Li$^+$ was present, the concentration of Na$^+$ in PBS was reduced appropriately so as to maintain a constant ionic strength. Incubations were terminated by aspirating the PBS and replacing it with 1 ml of HClO$_4$ (5%, v/v).

Abbreviations used: PtdIns, phosphatidylinositol; PtdIns4P, phosphatidylinositol 4-phosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; Ins$P$, inositol monophosphate; InsP_2, inositol bisphosphate; InsP_3, inositol trisphosphate; InsP_4, inositol tetrakisphosphate; Ins1P, inositol 1-phosphate; Ins(1,4)P_2, inositol 1,4-bisphosphate; Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; Ins(1,3,4)P_3, inositol 1,3,4-trisphosphate; Ins(1,3,4,5)P_4, inositol 1,3,4,5-tetrakisphosphate; PBS, phosphate-buffered salt solution (for composition, see the preceding paper [13]); ACTH, adrenocorticotropin.

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Extraction and separation techniques

After the termination of experimental incubations, cells were scraped from the plastic substratum of the dish with a rubber policeman in the presence of 2 mg of bovine serum albumin (fraction 5). The entire acid extract was transferred to a small glass tube and the Petri dishes were rinsed with 1 ml of $HClO_4$ (1%, v/v). The combined acid extract was centrifuged at 2000 g for 5 min and the protein pellet retained for lipid extraction. The supernatant was neutralized with 1.5 M-KOH containing 75 mM-Hepes in the presence of universal indicator. $KClO_4$ was precipitated at 0 °C for 2 h and removed by brief centrifugation. The neutralized acid extracts were diluted to 12 ml with 5 mM-$Na_2B_4O_7$/ 0.5 mM-EDTA, and inositol phosphates were separated by ion-exchange chromatography on Dowex 1 (X10, 100–200 mesh; formate form) as described previously [7,20]. We have found that the extraction procedure described above routinely yields results identical with those obtained with the trichloroacetic acid extraction described by others [21]. However, our method is very much simpler and does not necessitate repeated extractions with diethyl ether to remove the trichloroacetic acid.

The $HClO_4$-precipitated protein pellet was washed with 1 ml of water and the lipids extracted as described previously [4]. Lipid extracts were dried, re-suspended in 0.5 ml of chloroform and deacylated by alkaline hydrolysis [4]. The water-soluble (and ^{3}H-labelled) products of this deacylation were separated by anion-exchange chromatography as described previously [4].

Chemicals

Synthetic vasopressin analogues used in the present study were generously given by Professor M. Manning, Medical College of Ohio, Toledo, OH, U.S.A., and are listed in the preceding paper [13]. Other peptides and receptor agonists were from Penninsula Laboratories, Sigma or Bachem. myo-[2-^{3}H]Inositol was from New England Nuclear. Other chemicals were of the highest grade available and from the sources given previously [4,5,7,13].

RESULTS AND DISCUSSION

Vasopressin-stimulated inositol phosphate accumulation and inositol-lipid depletion in WRK 1 cells

When [^{3}H]Ins-labelled WRK 1 cells were stimulated with 0.2 μM-vasopressin, there was a rapid accumulation of [^{3}H]InsP_2 and [^{3}H]InsP_3, but [^{3}H]Ins$P$ accumulation was delayed for at least 2 min (Figs. 1 and 2). The accumulation of labelled InsP_2 and InsP_3 was statistically significant after 3 s exposure to the hormone ($P < 0.01$) and was associated with a diminution of the radioactivity in the polyphosphoinositides. The relative latency of vasopressin-stimulated Ins$P$ accumulation (and PtdIns depletion) in WRK 1 cells provides further evidence that the primary hormone-stimulated event in these, as in other cells ([14,15]; see refs. [1,2] for reviews), is the breakdown of PtdIns4P and/or PtdIns(4,5)P_2. The present results do not indicate whether the hormone stimulates the breakdown of both these lipids or merely the latter, with InsP_2 accumulating as a result of monoesterase attack upon InsP_3 and PtdIns4P becoming depleted due to the resynthesis of PtdIns(4,5)P_2. In either
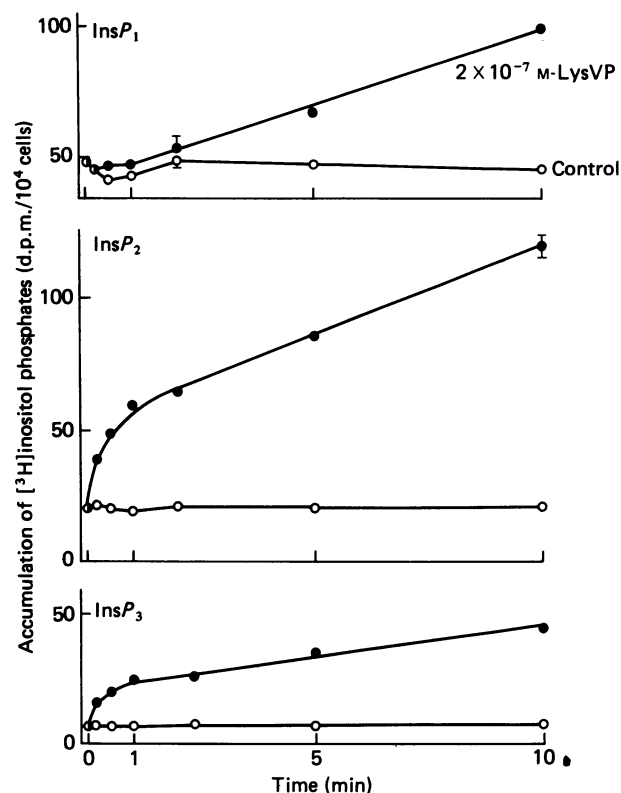

Fig. 1. Accumulation of [^{3}H]inositol phosphates in WRK 1 cells: influence of long-term stimulation with vasopressin

[^{3}H]Inositol-labelled WRK 1 cells were washed free of culture medium and bathed in PBS containing 10 mM-LiCl. [8-lysine]Vasopressin (LysVP) (2×10^{-7} M, ●) or control additions (○) were made 10 min later ($t = 0$ in the Figure) and incubations were terminated at the times indicated as described in the Materials and methods section. Radioactivity recovered in individual inositol phosphates was determined after anion-exchange chromatography. The radioactivity present in the total inositol lipids of unstimulated cells was 1221 ± 46 d.p.m./10^4 cells ($n = 6$). Results are means ± S.E.M. of three determinations from a single experiment, typical of three. Where no error bars are shown, these are contained within the symbols.

case, it seems most likely that PtdIns is ultimately depleted as a consequence of its utilization in polyphosphoinositide resynthesis and that Ins$P$ accumulates as a result of phosphomonoesterase attack upon InsP_2 and InsP_3.

The initial rate for the accumulation of [^{3}H]InsP_2 and [^{3}H]InsP_3 in vasopressin-stimulated cells was considerable: 4% of the radioactivity associated with all inositol lipids appeared in these products after 10 s stimulation with the hormone, although the radioactivity of the inositol lipids themselves fell by only 1% over the same time period. This represents an initial rate of inositol phosphate accumulation that is very much greater than that which we have previously observed in vasopressin-stimulated hepatocytes [5]. The discrepancy between the accumulation of radioactivity in inositol phosphates of stimulated cells and the loss of radioactivity from the parent lipids presumably reflects lipid resynthesis from the [^{3}H]inositol pool retained within the cell. Such compensatory resynthesis of inositol lipids after their receptor-mediated hydrolysis has previously been obser-
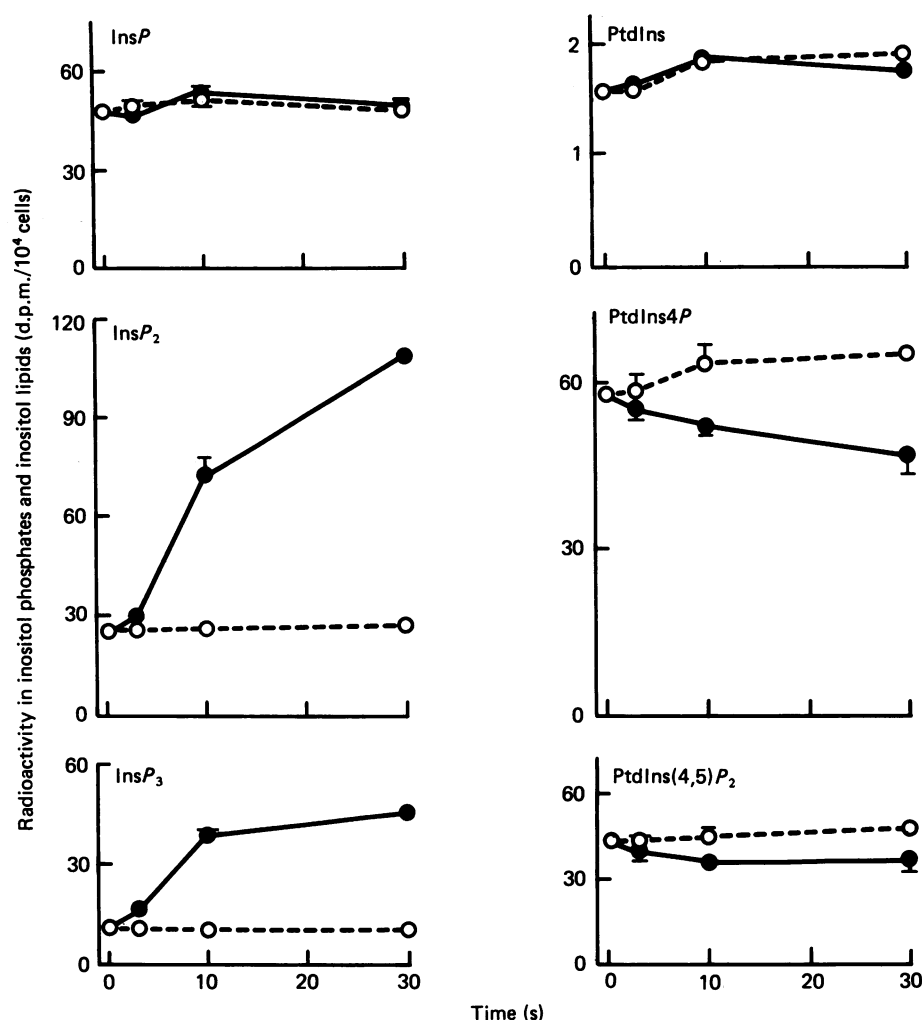
Fig. 2. Accumulation of [³H]inositol phosphates and depletion of [³H]inositol lipids in WRK 1 cells: influence of short-term stimulation with vasopressin

[³H]Inositol-labelled WRK 1 cells were washed free of culture medium and bathed in PBS containing 10 mM-LiCl. [8-lysine]Vasopressin (0.2 μM, ●) or control (○) additions were made 10 min later and incubations were terminated at the times indicated. Individual inositol phosphates and the deacylation products of the inositol lipids were extracted and separated by anion-exchange chromatography as described in the Materials and methods section. Results are means ± S.E.M. of three determinations from a single experiment, typical of three. Where no error bars are shown, these are contained within the symbols.

ved in other systems [4,14,15]. The availability of [³H]Ins is unlikely to have been rate-limiting to inositol-lipid resynthesis in the present study, since the radioactivity in the free inositol pool of unstimulated cells was four times that released in total inositol phosphates after 6 min stimulation with vasopressin (results not shown).

The results in Figs. 1 and 2 clearly indicate that vasopressin provokes an initial and rapid accumulation of InsP_2 and InsP_3 during the first 10–30 s of stimulation, which is followed by a slower, but sustained, accumulation of all three inositol phosphates. The diminished rate of accumulation of InsP_2 and InsP_3 after longer periods of stimulation is not merely a consequence of the hydrolysis of these compounds to Ins$P$, since insufficient radioactivity appears in this fraction to account for the decreased accumulation of [³H]InsP_2 and [³H]InsP_3. It is possible that these kinetics reflect an initial hydrolysis of a hormone-sensitive pool of PtdIns(4,5)P_2, whose further breakdown is limited by the

rate at which it can be replenished from the PtdIns and PtdIns4P reserves of the cell.

While this paper was in an advanced stage of preparation, Koreh & Monaco [46] reported that vasopressin provokes the depletion of PtdIns(4,5)P_2 and the associated accumulation of inositol phosphates in WRK 1 cells. The results of these workers also revealed that 60% of the cellular PtdIns(4,5)P_2 pool, and the entire complement of this lipid sensitive to hormone-stimulated hydrolysis, is synthesized from the relatively small proportion (17%) of the cellular pool of PtdIns, that is also sensitive to vasopressin-stimulated depletion. This result confirms previous evidence, and that reported herein, that points to the existence of a discrete, hormone-sensitive, pool of inositol phospholipids in WRK 1 cells [11,12].

The concentration-dependence of the vasopressin-stimulated accumulation of inositol phosphates in WRK 1 cells is shown in Fig. 3. The concentration of
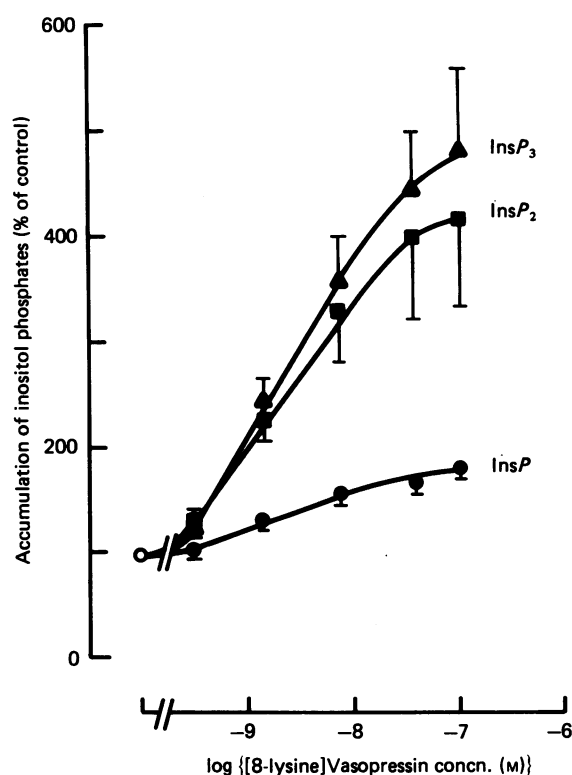
Fig. 3. Concentration-dependence of vasopressin-stimulated inositol phosphate accumulation in WRK 1 cells

[^{3}H]Inositol-labelled WRK 1 cells were rinsed free of culture medium and stimulated with various concentrations of vasopressin in the presence of 10 mM-LiCl as described in the legend to Fig. 1. Incubations were terminated after 6 min stimulation and inositol phosphates were separated as described in the text. The accumulation of Ins$P$ (●), InsP_2 (■) and InsP_3 (▲), calculated as a percentage of that observed in unstimulated cells, is expressed as means±S.E.M. from three to six separate experiments.

vasopressin required to provoke half-maximal accumulation of all three inositol phosphates was of the order of 3 nM. This is very similar to both the affinity of the WRK 1 cell V_{1a}-receptor for vasopressin [13], and also to the concentration of the hormone required to cause half-maximal inositol lipid depletion and/or inositol phosphate accumulation in a number of other tissues [4,7,14–17].

The relationship between the concentrations of a variety of vasopressin analogues required to provoke half-maximal accumulation of inositol phosphates in WRK 1 cells and those required to half-saturate WRK 1 cell V_{1a}-receptors is shown in Fig. 4. Also shown in this Figure is the relationship between the concentrations of various vasopressin antagonists that were required to half-maximally inhibit inositol phosphate accumulation and receptor occupation by a sub-optimal concentration of [8-lysine]vasopressin. The influence of antagonists upon vasopressin-induced inositol-phosphate accumulation was investigated in experiments similar to those shown in Fig. 5. The relative potencies of the ligands in Fig. 4 provides further evidence that the vasopressin receptors on WRK 1 cells are of the V_{1a} subtype [13,22]. There was a close correlation between receptor occupa-

tion by these peptides and their ability to influence inositol phosphate accumulation. This indicates that there is no 'receptor reserve' for V_{1a} receptor-mediated inositol phosphate accumulation in these cells. We have previously argued that the absence of a receptor reserve for agonist-induced inositol lipid depletion in several tissues is compatible with the notion that receptor-mediated inositol lipid breakdown is involved in the
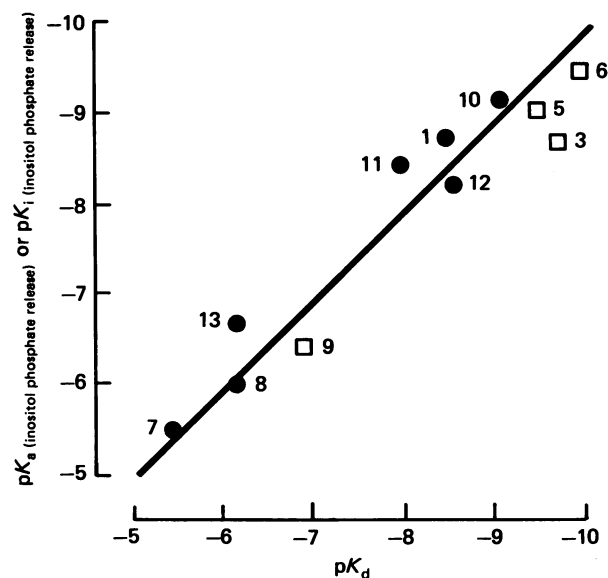

Fig. 4. Influence of vasopressin analogues on receptor occupation and inositol phosphate production in WRK 1 cells

[^{3}H]Inositol-labelled WRK 1 cells were rinsed free of culture medium and incubated for 10 min in the presence of 10 mM-LiCl. The cells were then exposed to various concentrations of vasopressin agonists alone (●) or to antagonists plus a fixed, suboptimal (10 nM), concentration of [8-lysine]vasopressin (□) in the continued presence of LiCl. Incubations were terminated 6 min later. The reciprocal logarithm (−log) of the concentrations of agonists required to provoke half-maximal inositol phosphate release [pK_a (inositol phosphate release)], or of antagonists necessary to reduce by 50% the release of inositol phosphates provoked by 10 nM-[8-lysine]vasopressin [pK_i (inositol phosphate release), see Fig. 5] were determined graphically. Dissociation constants (pK_d, i.e. −log K_d) for the various peptides were determined in unlabelled WRK 1 cells incubated with 10 nM-[^{3}H][8-lysine]vasopressin as described in the preceding paper [13]. The slope of the line depicted in the Figure is 1. Individual peptides are numbered as in the preceding paper [13] and are as follows; 1, [8-arginine]vasopressin; 3, des-9-glycine-[1-(β-mercapto-$\beta\beta$-cyclopentamethylenepropionic acid), 8-arginine]vasopressin; 5, [1-(β-mercapto-$\beta\beta$-cyclo-pentamethylene propionic acid),2-O-ethyltyrosine,4-valine,8-arginine]vasopressin; 6, des-9-glycine[1-(β-mercapto-$\beta\beta$-cyclopentamethylenepropionic acid),2-O-ethyltyrosine, 4-valine,8-arginine]vasopressin; 7, [8-arginine,9-glycine]-vasopressin; 8, [4-valine,8-D-arginine]vasopressin; 9, 1-deamino-[4-valine,8-D-arginine]vasopressin; 10, [1-(L-2-hydroxy-3-mercaptopropanoic acid),8-arginine]vaso-pressin; 11, [8-lysine]vasopressin; 12, [8-arginine]vasotocin; 13, oxytocin. Values for the various parameters are means of at least three separate determinations.
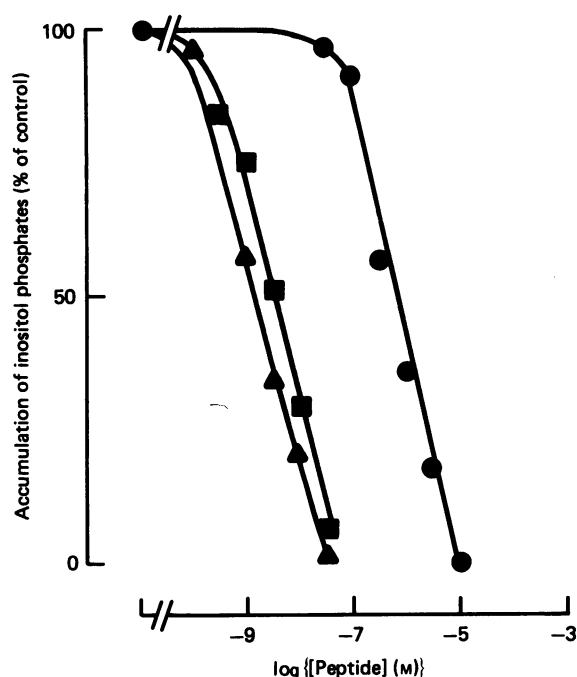
Fig. 5. Determination of K_i for the inhibition of vasopressin-stimulated inositol phosphate accumulation in WRK 1 cells

myo-[2-^{3}H]inositol-labelled WRK 1 cells were stimulated with 10 nM-[8-lysine]vasopressin in the presence of various amounts of 1-deamino-[4-valine,8-D-arginine]vasopressin (●), [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-ethyltyrosine, 4-valine, 8-arginine]vasopressin (■) or des-9-glycine-[1-(β-mercapto-β,β-cyclopenta-methylenepropionic acid), 8-arginine]vasopressin (▼). Inositol phosphate accumulation in the presence of antagonist is expressed as a percentage of that observed in antagonist-free controls. The inhibition constant (K_i) for each antagonist was calculated as:

$$K_i = IC_{50}/[1+([VP]/K_a)]$$

where IC_{50} is the antagonist concentration provoking a 50% inhibition of the vasopressin-induced response, [VP] is the concentration of [8-lysine]vasopressin present in the assay (10 nM) and K_a is the concentration of [8-lysine]vasopressin which evokes a half-maximal stimulation of inositol phosphate accumulation. Results are means of duplicate determinations in a single experiment typical of three to six which were averaged to give the data shown in Fig. 4.

mechanism of signal transduction at the plasma membrane [4,14,15,23]. The present results provide further support for this idea.

Influence of [Li$^+$] upon vasopressin-stimulated inositol phosphate accumulation in WRK 1 cells

As has been found in other tissues, Li$^+$ enhances the accumulation of inositol phosphates in vasopressin-stimulated WRK 1 cells (Fig. 6). This effect has generally been attributed to the ability of Li$^+$ to inhibit Ins1P phosphatase [24,25]. However, in WRK 1 cells, the accumulation of all three inositol phosphates was enhanced in the presence of Li$^+$. Half-maximal accumulation of Ins$P$ in vasopressin-stimulated cells was observed with 0.9 mM-Li$^+$. Maximal accumulation of Ins$P$ occurred with 3 mM-Li$^+$, and the accumulation of this product
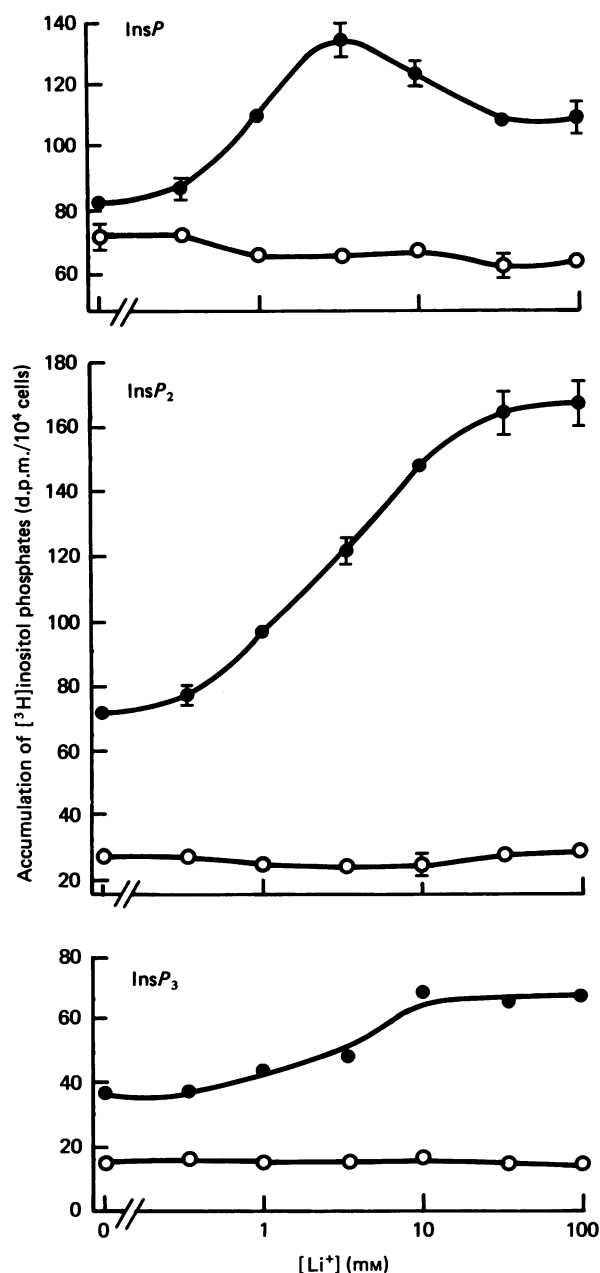

Fig. 6. Influence of [Li$^+$] on vasopressin-stimulated inositol phosphate accumulation in WRK 1 cells

[^{3}H]Inositol-labelled WRK 1 cells were washed free of culture medium and bathed in PBS containing various concentrations of LiCl. [8-lysine]Vasopressin (2×10^{-7} M, ●) or control additions (○) were made 10 min later and incubations were terminated after a further 6 min. The radioactivity recovered in individual inositol phosphates was determined after anion-exchange chromatography as described in the Materials and methods section. The radioactivity recovered in the total inositol lipids of unstimulated cells was 1800 ± 92 d.p.m./10^4 cells ($n = 6$). Results are means $\pm$ S.E.M. of three determinations from a single experiment, typical of three. Where no error bars are shown, these are contained within the symbols.

declined in the presence of greater concentrations of the ion. Half-maximal accumulation of InsP_2 and InsP_3 was observed with 3.0 and 3.6 mM-Li$^+$, whereas maximal accumulation of these products occurred in the presence

Table 1. Agonists that failed to influence inositol phosphate accumulation in WRK 1 cells

WRK 1 cells were cultured with myo-[2-^{3}H]inositol and incubated in the presence of 10 mM-Li$^+$ as described in the text. Agonists were added to give the final concentrations indicated. Incubations were terminated 6 min later and inositol phosphates separated and assayed for radioactivity as described in the text. All agonists were tested for inositol-phosphate-releasing activity in at least two separate experiments.

Agonist	Maximum concentration tested (M)
ACTH-(1–24)-peptide	10^{-5}
Adenosine	10^{-5}
Angiotensin	10^{-6}
Carbamylcholine	10^{-5}
Cholecystokinin	10^{-5}
Dopamine (3,4-dihydroxyphenethylamine)	10^{-5}
β-Endorphin	10^{-5}
[Leu]Enkephalin	10^{-5}
Gastrin	2×10^{-6}
Histamine	10^{-5}
Luliberin (luteinizing-hormone-releasing hormone)	10^{-5}
α-Melanotropin (melanocyte-stimulating hormone)	10^{-5}
Noradrenaline	10^{-5}
Prolactin	6×10^{-6}
Somatostatin	10^{-5}
Substance P	10^{-5}
Vasoactive intestinal peptide	2×10^{-6}

Table 2. Agonist-stimulated inositol phosphate accumulation in WRK 1 cells

WRK 1 cells were cultured with myo-[2-^{3}H]inositol and incubated in the presence of 10 mM-Li$^+$ as described in the text. Agonists were added to give the final concentrations indicated. Incubations were terminated 6 min later and inositol phosphates separated and assayed for radioactivity as described in the text. Results are means$\pm$S.E.M. of four to ten observations from two to five separate experiments.

Agonist	Concn. (μM)	Total [^{3}H]inositol phosphates accumulated (% of control)
[8-lysine]Vasopressin	0.2	296 ± 22
Bradykinin	0.1	231 ± 9
5-Hydroxytryptamine	10	167 ± 14

of 30 and 10 mM-Li$^+$ respectively. The diminution of vasopressin-stimulated Ins$P$ accumulation in the presence of Li$^+$ at a concentration greater than 3 mM presumably reflects the trapping of released inositol phosphate headgroups as InsP_2 and InsP_3. This provides further evidence that the accumulation of Ins$P$ in stimulated WRK 1 cells occurs, at least in part, as a consequence of phosphomonoesterase attack upon InsP_2 and InsP_3.

The inositol phosphates are dephosphorylated by a number of separate phosphomonoesterases [26–31]. In liver, at least, these include both membrane-bound and soluble activities [26,29]. The observation that vasopressin-stimulated InsP_2 accumulation is greatly increased by Li$^+$ in WRK 1 cells suggests that the dephosphorylation of this product may occur mainly via the Ins(1,4)P_2 4-phosphatase route, which we have found to be cytosolic and sensitive to inhibition by Li$^+$ in the liver (A. Morris, D. Storey, R. H. Michell & C. J. Kirk, unpublished work).

The ability of Li$^+$ to enhance InsP_3 accumulation in WRK 1 cells and hepatocytes contrasts with the failure of this ion to influence InsP_3 5-phosphatase activity in hepatocytes [26] or platelets [31]. However, it is now clear that two isomers of InsP_3 accumulate in stimulated cells. The product of PtdIns(4,5)P_2 hydrolysis would be expected to be Ins(1,4,5)P_3, but Ins(1,3,4)P_3 is the predominant isomer found in parotid gland [32], hepatocytes [5], HL60 cells and pancreatic acinar cells [33] that have been stimulated for more than a few seconds. Our own preliminary results indicate that Ins(1,3,4)P_3 is the major InsP_3 isomer to accumulate in WRK 1 cells that have been stimulated with vasopressin for 6 minutes (C. J. Barker, S. McCallum & C. J. Kirk, unpublished work). Ins(1,3,4)P_3 is probably derived from a novel InsP_4, Ins(1,3,4,5)P_4, which has recently been shown to accumulate in ligand-stimulated brain slices [34], hepatocytes [5] and WRK 1 cells (C. J. Barker, S. McCallum & C. J. Kirk, unpublished work). The ability of Li$^+$ to enhance vasopressin-stimulated InsP_3 accumulation in WRK 1 cells could reflect an ability of this ion to inhibit the activity of Ins(1,3,4)P_3-specific phosphomonoesterases.

Influence of other agonists on inositol phosphate accumulation in WRK 1 cells

Apart from vasopressin, a large number of other agonists were screened for their ability to provoke inositol phosphate accumulation in WRK 1 cells. Many of these were without effect (Table 1), but bradykinin, and 5-hydroxytryptamine did cause significant accumulation of inositol phosphates in these cells (Table 2). Both these agonists have previously been shown to stimulate PtdIns(4,5)P_2 depletion and/or inositol phosphate accumulation in other tissues [35,36] and it seems likely that these hormones provoke receptor-mediated hydrolysis of PtdIns(4,5)P_2 in WRK 1 cells too.

The concentration-dependence of bradykinin-stimulated inositol phosphate accumulation in WRK 1 cells is shown in Fig. 7. Half-maximal accumulation of InsP_2 and InsP_3 was observed in the presence of about 5 nM-bradykinin. This is very similar to the concentration of bradykinin required to half-saturate the bradykinin receptors of intestinal smooth muscle and mucosal cells [37,38], which suggests that, as we have previously argued for vasopressin receptors [4,14,15], PtdIns(4,5)P_2 breakdown may be closely coupled to bradykinin-receptor occupation.

Also shown in Fig. 7 is the influence of sub-maximal and maximal concentrations of vasopressin upon the accumulation of InsP_2 and InsP_3 provoked by a range of concentrations of bradykinin in WRK 1 cells. In the presence of concentrations of vasopressin that were themselves sufficient to provoke only a modest accumulation of inositol phosphates, the effects of the two hormones appeared to be virtually additive. However, when the cells were exposed to a concentration of
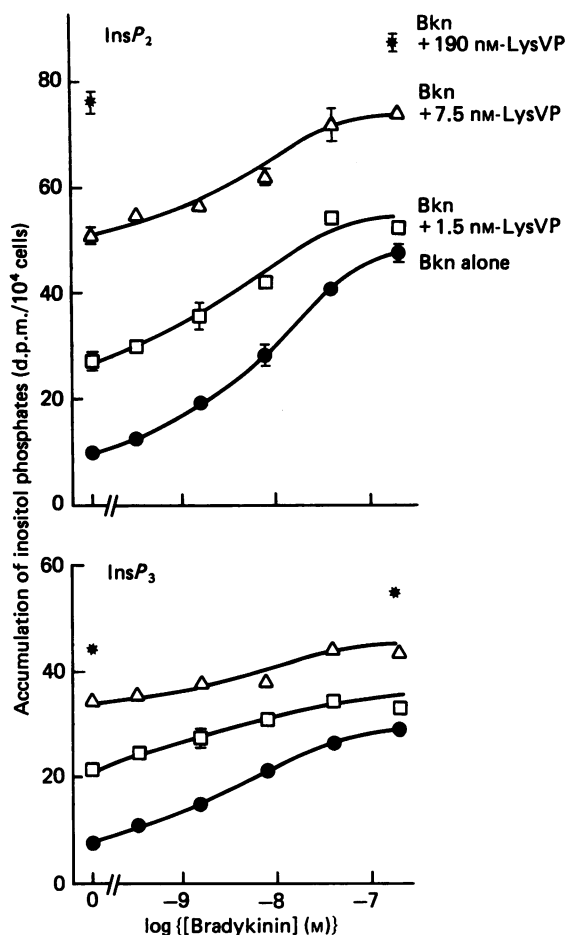
Fig. 7. Additivity of vasopressin (LysVP)- and bradykinin (Bkn)-stimulated inositol phosphate accumulation in WRK 1 cells

[³H]Inositol-labelled WRK 1 cells were washed free of culture medium and bathed in PBS containing 10 mM-LiCl. After 10 min, the cells were exposed to various concentrations of either bradykinin alone (●) or bradykinin plus 1.5 nM- (□), 7.5 nM- (△) or 190 nM- (✻)[8-lysine]vasopressin. Incubations were terminated after 6 min stimulation and the radioactivity recovered in $InsP_2$ and $InsP_3$ was determined after anion-exchange chromatography as described in the text. Results are means±s.e.m. of three determinations from a single experiment, typical of two. Where no error bars are shown, these are contained within the symbols.

vasopressin sufficient to cause maximal vasopressin-induced inositol phosphate production, bradykinin (0.2 μM) caused little further accumulation of these products. These results suggest that the two hormones share at least some common elements in the mechanism(s) by which they hydrolyse inositol lipids in WRK 1 cells, and that one such element becomes saturated in the presence of maximal concentrations of both hormones. The saturable element could be the GTP-dependent coupling protein, which appears to couple the activated receptor to a membrane-bound inositol-lipid phosphodiesterase in WRK 1 [39] and other cells [40–43], this enzyme or the availability of the inositol lipid itself.

The present results clearly demonstrate that WRK 1 cells carry vasopressin receptors of the V_{1a} subtype and

receptors for other hormones which are coupled to the hydrolysis of membrane inositol phospholipids. It seems likely that the primary consequence of receptor activation is the hydrolysis of $PtdIns(4,5)P_2$ to yield $InsP_3$ and diacylglycerol. Both these products have been suggested to fulfil 'second-messenger' roles within the cell. $InsP_3$ causes Ca^{2+} mobilization from an intracellular store (see ref. [2] for review) and diacylglycerol is a physiological activator of protein kinase C (see ref. [44] for review). These two intracellular messengers act synergistically to provoke the phosphorylation of a variety of enzymes in target cells [2,44,45]. The physiological consequences of the release of these messengers inside WRK 1 cells is not yet clear, but they may be involved in the stimulation of protein and lipid synthesis observed in the presence of vasopressin [8,9].

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